TRIGGERS FOCUS GROUP
RECOMMENDATION #8

Toxicity Tests with Controls that did not Meet US EPA Test Acceptability Criteria for NPDES Testing
January 17, 2007

OBJECTIVE: The objective of this requirement is to provide guidance regarding how to interpret toxicity data from tests with Lab Controls that did not meet the US EPA test acceptability criteria (TAC).

PROBLEM STATEMENT: The agricultural community has formed Coalitions for their atersheds so that they have a logical geographical boundary for monitoring, as well as a group of growers to pay for the monitoring. The watersheds range from relatively small (e.g., a water district with 3-5 samples per sampling event) to very large (e.g., Sacramento Valley with ~20 sites per sampling event); most Coalitions are monitoring anywhere from 8 to 12 events per year. Toxicity testing for these Coalitions includes acute *Ceriodaphnia*, acute fathead minnow, and the chronic *Selenastrum* tests.

The following TAC have been established for toxicity testing in the EPA test methods manuals:

Test	EPA Manual Test Acceptability Criteria (TAC)
Acute *Ceriodaphnia*	≥90% survival in the Control treatment
Acute Fathead Minnow	≥90% survival in the Control treatment
Chronic *Selenastrum*	mean cell density of 200,000 cells/mL (when tested w/o EDTA) and CV <20% at the Control treatment.

The manual indicates that if the test acceptability criteria are not met, the test must be repeated with a newly collected sample. The initial statement made in both the acute and chronic manuals (Section 1.1) indicate, “This manual describes acute (or chronic) toxicity tests for use in the National Pollutant Discharge Elimination System (NPDES) Permit Program”. The test conditions and test acceptability criteria within these manuals were established with this in mind. However, the logistical and regulatory framework inherent to the ILP monitoring is very different from most (if not all) NPDES testing situations. For example, re-collection of a sample for a test that does not meet USEPA TAC would typically occur at a single “point source” discharge in most NPDES cases, whereas re-collection of samples under an ILP monitoring program could require the re-collection of samples from multiple sites.

This recommendation to the TIC describes actions that should be taken when a control treatment does not meet the USEPA TAC. It should be noted that experienced, California Environmental Laboratory Accredited, toxicity testing laboratories should be capable of meeting the EPA TAC most of the time. This is reflected in the program completeness criteria (Completeness of ≥90% as defined in the ILP QAPP Guidelines).

It is possible to determine that a test sample is not toxic even when a Control treatment fails to meet TAC. Toxicity is defined as a statistically significant negative effect; if there is no such negative effect, then the water sample is not considered toxic, assuming that the testing was performed following the EPA method.

- For the **acute survival tests**, the water samples often exhibit 90 -100% survival. In these cases, the absence of a negative effect in the water sample indicates that there is no toxicity (at least none that can be detected with an acute test exposure).

- For the **chronic algal growth test**, the water samples often exhibit algal growth that is markedly greater than the Control treatment response, again indicating that there is no toxicity.

RECOMMENDATION:

Decision Step 1: If the Control treatment meets all US EPA TAC, then proceed to statistical analyses for determination of the presence of statistically significant reductions in organism survival or algal growth. For samples that exhibit toxicity, the follow-up requirements in the ILP MRP must be followed.

Proposed Decision Step 2a: If the control exhibits <90% survival, an acute test of a water sample exhibits 90-100% survival, and the program completeness standard for the test is met (e.g., ≥90% of testing performed successfully to meet ILP Completeness Objective), no further testing is required and the test result should be "flagged" to denote <90% survival in the Control treatment.

If an acute test of a water sample exhibits 90-100% survival, and the program completeness objective for the test is not met, then a re-test of the original sample must be initiated within 24 hours of the observation of a Control treatment with <90% survival. For the fathead minnow test, the laboratory must take the steps to procure test species within one working day, and the re-test must be initiated within one day of fish being available from a supplier. In all cases, both the original test results and the re-test results must be reported by the Coalition; the re-test results should be flagged to note that the re-test was initiated outside of the holding time limit. New samples must be collected within five working days of the laboratory identifying a second failure in TAC, if the re-test does not meet US EPA TAC.

Proposed Decision Step 2b: A water sample is not considered toxic if all of the following is true:
- The algal test control does not meet the US EPA TAC for variability (i.e., coefficient of variation >20%), and
- A water sample exhibits an algal cell density that is greater than the algal cell density in the control, and
- The average algal growth in the replicates does not overlap with that in the control (i.e., all test sample replicates exhibit greater algae growth than all control replicates), and

- The Program completeness objective is met.

If the program completeness objective for the test is not met, then a re-test of the original sample must be initiated within 24 hours of the termination of the initial algal test. In all cases, both the original test results and the re-test results must be reported by the Coalition; the re-test results should be flagged to note that the re-test was initiated outside of the holding time limit. New samples must be collected if the re-test does not meet US EPA TAC.

If an algal test Control treatment does not meet the minimum growth TAC of ≥ 20,000 cells/mL, then a retest of the original sample must be initiated within 24 hours of the termination of the initial algal test. Both the original test results and the re-test results must be reported by the Coalition; the re-test results should be flagged to note that the re-test was initiated outside of the holding time limit. New samples must be collected within five working days of the laboratory identifying a second failure in TAC, if the re-test does not meet US EPA TAC.

Proposed Decision Step 3: If a Control treatment does not meet US EPA TAC, and the associated ambient water sample(s) have <90% survival (for an acute toxicity test) or the algal growth is less than the Control, then the Regional Board will be notified within 1 business day of the observation of the results in question so that an agreement can be reached regarding how to proceed. At a minimum, re-testing of the original sample within 24 hours of the observed test failure will be required and test results should be "flagged". For the fathead minnow test, the laboratory must take the steps to procure test species within one working day, and the re-test must be initiated within one day of fish being available from a supplier. If re-testing does not begin within 24 hours, then re-sampling must be conducted within 48 hours of the observed test failure. Re-test results should be flagged to note that the re-test was initiated outside of the holding time limit. New samples must be collected within five working days of the laboratory identifying a second failure in TAC, if the re-test does not meet US EPA TAC.

Note: it is important to recognize that when re-testing a sample beyond the 36-hour holding time prescribed in the test method manual, there is a possibility that toxicity will be reduced or completely gone. In addition, when re-sampling at a site, the new sample does not represent the same conditions under which the original sample was collected (this is particularly important to note when sampling is meant to characterize a specific event such as stormwater runoff).

The reporting of data that do not meet US EPA TAC must also include an assessment from the laboratory as to what may have caused the test control performance issue, the laboratory's corrective measures to prevent future control failures, a comparison of the data against the EPA test performance measures, and a comparison of the data against the ILP required completeness criteria in the Coalition's QAPP.